Makoto Nishida*, Shiro Kurita* & Shigeya Niizeki**:

# Cytotaxonomy of Ophioglossales II.*** Chromosome number in *Sceptridium*

西田誠*・栗田子郎*・新関滋也**: ハナヤスリ目の細胞分類学 II***
オオハナワラビ属の染色体数

(Pl. IV—V)

Among rather scarce investigators of *Sceptridium*, Britton (1953) and Wagner (1955) found n=45 in *S. dissectum* Spring complex in America, Wagner (1955) observed the same gametic number in *S. multifidum* and in *S. oneidense*, and Manton and Sledge (1954) reported that *S. daucifolium* in Ceylon is a tetraploid species of which the number is n=90, as is summarized in Tab. 1.

Since Nishida (1960) recognized five species including two varieties in Japan, we examined chromosome in them all but one, following the method as described previously (Niizeki, Nishida and Kurita 1963). Names and localities of the material plants are in the text and Tab. 1.

## Observations

*Sceptridium ternatum* (Thunb.) Lyon. Material was collected at Godai, Chiba Pref. In metaphase of the first meiotic division and in late diakinesis we distinctly counted 45 bivalent chromosomes (Fig. 1). The meiosis went on normally, and produced tetrads and seemingly available spores. Okuno (1936), however, counted 44 gametic chromosomes in this species.

*Sceptridium ternatum* var. *nippocicum* (Makino) Nishida Material was from Narita, Chiba Prefecture and from Ashikaga, Tochigi Prefecture. The gametic chromosomes were also 45 (Fig. 2), and we could not distinguish this variety cytologically from its mother species.

*Sceptridium multifidum* (Gmel.) Nishida This is a mountainous species distributed in northern Japan. Specimens from Mt. Akagi and Nikko, Gunma Pref., clearly showed 45 bivalent chromosomes (Fig. 3), as American specimens studied by Wagner (1955).

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*Sceptridium multifidum* (Gmel.) Nhishida var. *robustum* (Rupr.) Nishida Material was collected at Antaroma and Sapporo, Hokkaido. In the first meiotic division, 45 bivalent chromosomes were counted (Fig. 4). Each of which at diakinesis ranged from 3 to 6 μ in length as in *S. multifidum.*

*Sceptridium japonicum* (Plantl) Lyon This species, mainly distributed in Eastern Asia, is interesting in chromosome constitution, as it is hexaploid. Specimens from some localities in Chiba Prefecture were examined. In many spore mother cells there were 135 bivalent chromosomes (Fig. 5). This is the only example of hexaploid in Botrychiaceae so far known.

*Sceptridium daucifolium* (Wall.) Lyon The plants were brought from Yakushima Island by Mr. Kawabata and have been cultivated in our green house. The chromosome number of this species was already reported by Manton and Sledge (1954). They studied the material from Ceylon and found 90 bivalent chromosomes in meiosis. We also counted n=90 in the present material. The bivalents in late diakinesis ranged from 2 to 5 μ in length, being somewhat smaller than those in other species of the genus. It is worth noting that three or four, loosely paired, dumbbell-shaped chromosomes were observed in this species. No such could be found in other species of *Sceptridium,* although a similar pattern has already been observed in *Japanobotrychium* (Niizeki, Nishida and Kurita, 1963).

## Discussion

Nishida (1960) divided the genus *Sceptridium* into two sections, Sect. *Sceptridium* (=Sect. *Elongatae* in Subgen. *Sceptridium* in the genus *Botrychium* sensu Clausen 1938) and Sect. *Multifida* (=Sect. *Multifidae* in Subgen. *Sceptridium* in the genus *Botrychium* sensu Clausen 1938). So far as we know, all of the members belonging to Sect. *Multifida* have 45 chromosomes in haploid phase, and they are diploid plants. Among the species belonging to Sect. *Sceptridium,* those in New World have also 45 haploid chromosomes, while those in Asia are polyploid and have 90 or 135 chromosomes in haploid phase. We have counted 135 chromosomes in haploid phase of *Sceptridium japonicum.* This is the largest chromosome number and the plant is the only example of hexaploid in Botrychiaceae, in which n=45 or n=90 are common numbers. An hexaploid might be induced by hybridization from two plants which respectively have 45 and 90 chromosomes. However, this hardly can be the case with Botrychiaceae, for the

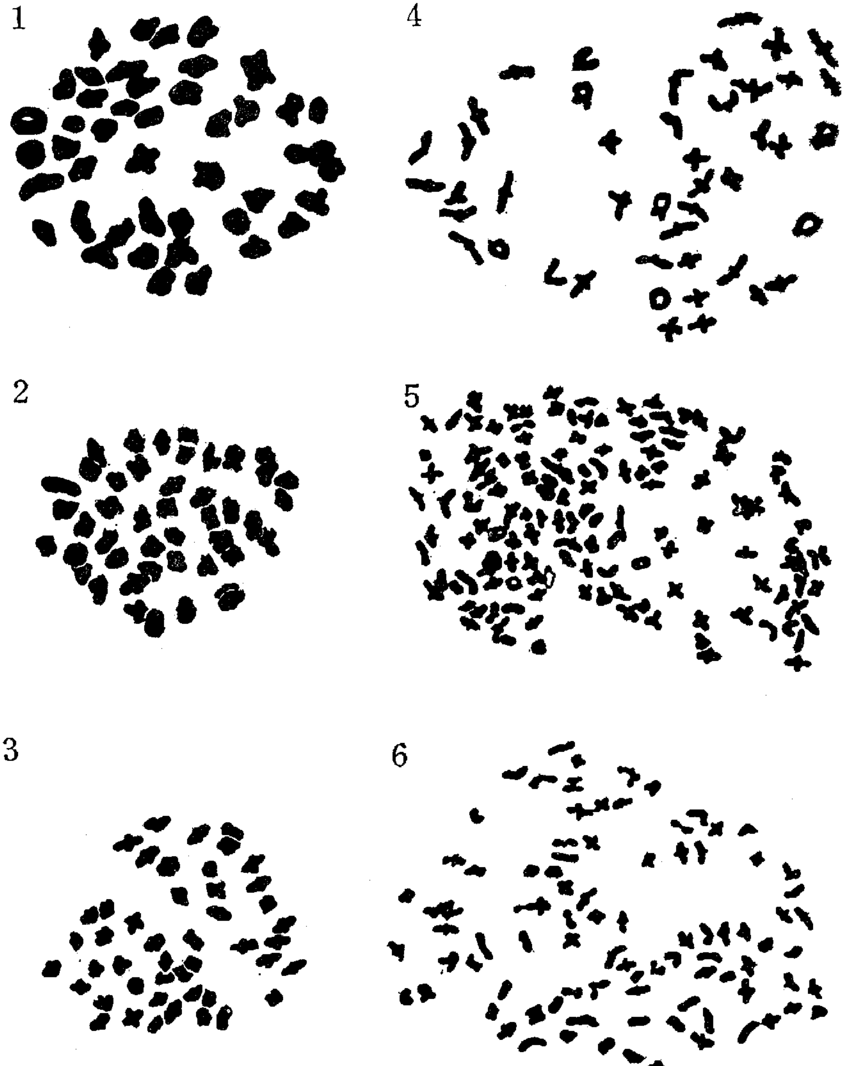

Fig. 1—6. Chromosomes of Japanese *Sceptridium*. 1. *S. ternatum*, n=45. × ca. 700. 2. *S. ternatum* var. *nipponicum*, n=45. × ca. 500. 3. *S. multifidum*, n=45. × ca. 500. 4. *S. multifidum* var. *robustum*, n=45. × ca. 500. 5. *S. japonicum*, n=135. × ca. 400. 6. *S. daucifolium*, n=90. × ca. 530.

gametophytes are subterranean in this family. Even if gametophytes were grown closely side by side, hardly any spermatozoid would travel through the soil. What would be the origin of the hexaploid, *S. japonicum*? Although we have no answer for this, we may refer to an interesting case in *Japanobotrychium strictum* (Niizeki, Nishida and Kurita, 1963) in which some archesporial cells had approximately 135 chromosomes. Such an incidental triploidy might sometimes be an origin of hexaploidy.

Tab. 1. Chromosome number of *Sceptridium*.

| Species | Locality | Chromosom number | Investigator |
|---|---|---|---|
| *S. ternatum* | Japan | n=44 | Okuno, 1936. |
| | Japan | n=45 | |
| —— var. *nipponicum* | Japan | n=45 | |
| *S. multifidum* | America | n=45 | Wagner, 1955. |
| | Japan | n=45 | |
| —— var. *robustum* | Japan | n=45 | |
| *S. japonicum* | Japan | n=135 | |
| *S. daucifolium* | Ceylon | n=90 | Manton & Sledge, 1954. |
| | Japan | n=90 | |

*S. ternatum* var. *nipponicum* represents the plant which once was described by Makino (1915) as a new species, *Botrychium nipponicum* Makino, as to be closely related to *S. japonicum*, but later Nishida (1959) confirmed that it is rather like *S. ternatum* than *S. japonicum* in gross morphology, and termed it as a variant of *S. ternatum*. Since *S. ternatum* and *B. nipponicum* are common in chromosome number 45 whereas *S. japonicum* is distinctive (n=135), it is almost certain that *B. nipponicum* should be placed close to *S. ternatum* and rather far from *S. japonicum*.

Another Japanese species, *S. minus* Nishida, could not be treated in this work. This species was formerly described by Hara (1933) as a variant of *S. japonicum*, but Nishida treated it as an independent species closely retated to *S. mutifidum* (Tagawa, 1958). Unfortunately we could not obtain fresh specimens of this species for the present purpose. For certain reasons as those for regarding *B. nipponicum* as a variant of *S. ternatum*, we might consider *S. minus* a variant of *S. multifidum*, as will be reported elswhere.

## Summary

Cytotaxonomical study on four species including two varieties of *Sceptridium* was done. The gametic chromosomes of 45 were distinctly counted in two species and two varieties, and n=90 was counted in *S. daucifolium*. *S. japonicum* was found to have n=135. This is the only example of hexaploid in Botrychiaceae so far known. These results are summarized in Tab. 1.

We are grateful to Mr. M. Kawabata and Mr. T. Sasaki who were very helpful in getting the material used in this work.

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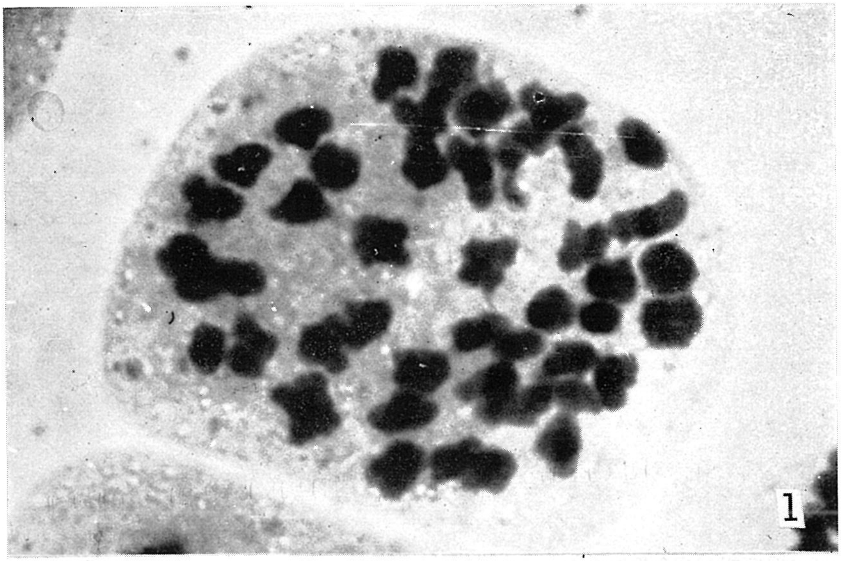
## Explanation of plates IV—V

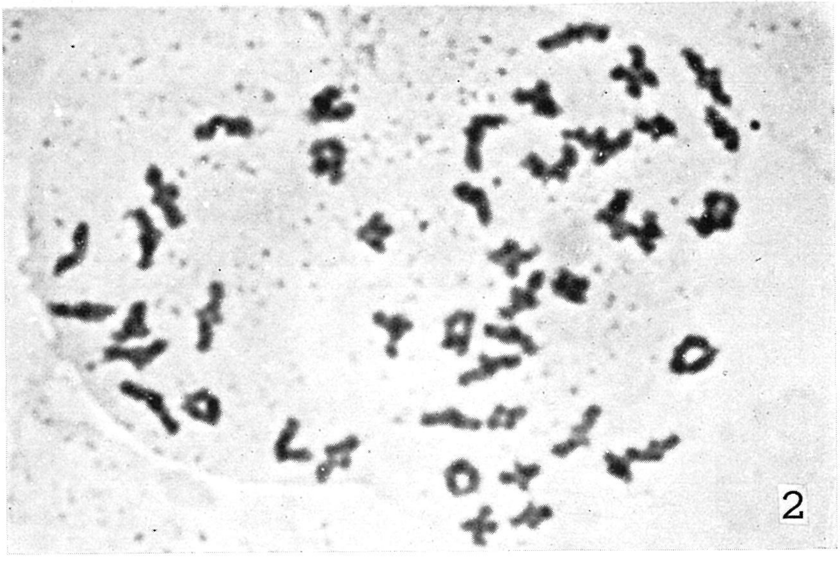
Plate IV. 1: *S. ternatum*, n=45. 2: *S. multifidum* var. *robustum*, n=45.

Plate V. 3: *S. japonicum*, n=135. 4: *S. daucifolium*, n=90.

## 摘　要

ウスイハナワラビを除く日本産のオオハナワラビ属の染色体数を観察した。フユノハナワラビ，アカハナワラビ，ヤマハナワラビ，およびエゾフユノハナワラビは n=45 の二倍体であった。オオハナワラビは n=135 で八倍体でるる。ハナワラビ科で六倍体を観察したのは筆者等が最初である。ハナワラビ科のような地中性の配偶体を形成するものでは交雑が起ることはほとんど考えられないから，この六倍体がいかにして生じたかが問題となる。これに対する明確な解答は得ていないが，筆者等(1963)がナガホノナツノハナワラビで観察した偶発的な三倍性胞原細胞形成のようなことがこの起源となりうるかもしれない。ホウライハナワラビは，すでに Manton and Sledge が報告したように n=90 であった。この種の核型は他種のそれと比べて特徴がある。すなわち，ナツノハナワラビ属でみられた小さな亜鈴形の二価染色体が 3 ないし 4 個みられ，他の二価染色体も比較的小さい。アカハナワラビは，かってオオハナワラビに近いものと考えられていたが，染色体数その他の点から考えてフユノハナワラビの変種とするのが妥当だろう。





M. NISHIDA, S. KURITA & S. NIIZEKI:
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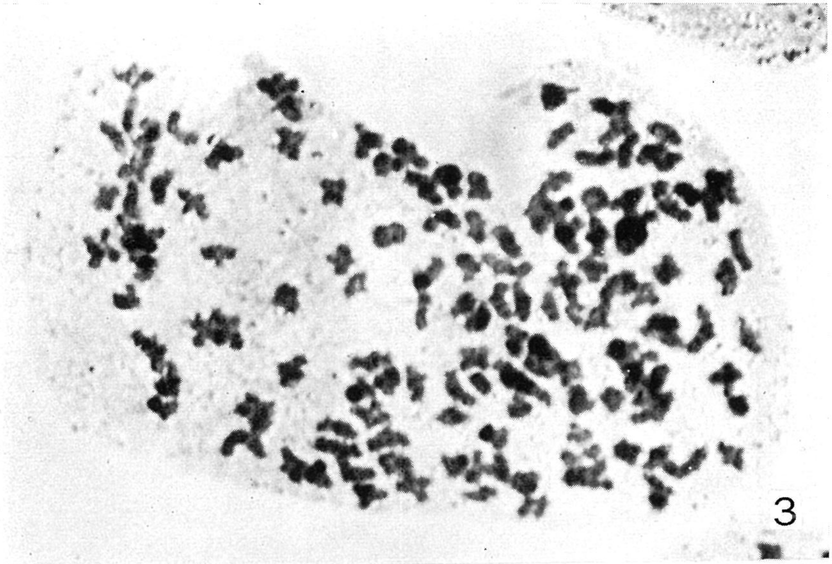

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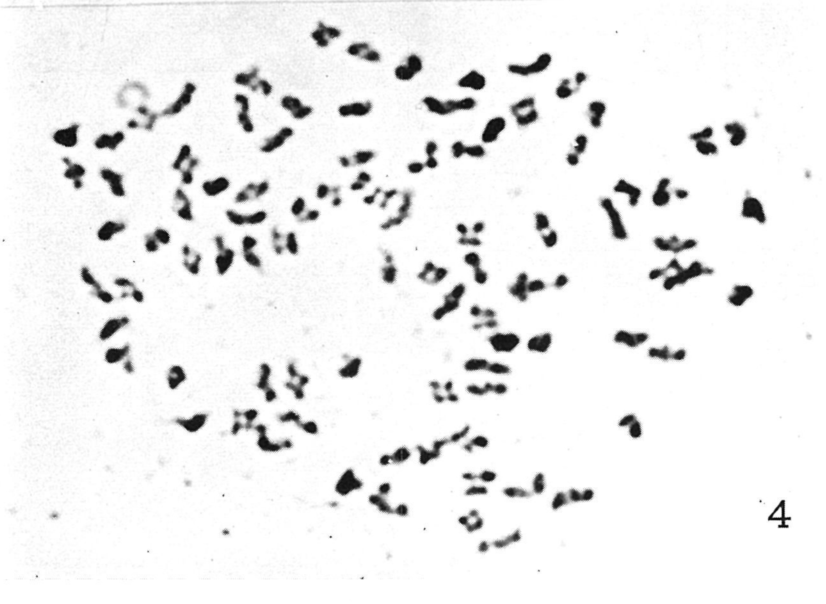

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M. Nishida, S. Kurita & S. Niizeki:
Chromosome in *Sceptridium*